

PTO/PCT Rec'd 03 JAN 2001

09/720952

Title of the inventionProcess for obtaining HMG-CoA reductaseinhibitors of high purity

This application is a 371 of PCT/IB99/01553 09/17/1999

Technical Field

10 Lovastatin, pravastatin, simvastatin, mevastatin, atorvastatin and derivatives and analogs thereof are known as HMG-CoA reductase inhibitors and are used as antihypercholesterolemic agents. The majority of them are produced by fermentation using microorganisms of different species identified as species belonging to *Aspergillus*, *15 Monascus*, *Nocardia*, *Amycolatopsis*, *Mucor* or *Penicillium* genus, some are obtained by treating the fermentation products using the method of chemical synthesis or they are the products of total chemical synthesis.

20 The purity of the active ingredient is an important factor for manufacturing the safe and effective pharmaceutical, especially if the pharmaceutical product must be taken on a longer term basis in the treatment or prevention of high plasma cholesterol. The accumulation of the impurities from the pharmaceuticals of lower purity may cause many 25 side effects during the medical treatment.

30 The present invention relates to a new industrial process for the isolation of HMG-CoA reductase inhibitors using so-called displacement chromatography. Use of the invention enables to obtain HMG-CoA reductase inhibitors of high purity, with high yields, lower production costs and suitable ecological balance.

Prior Art

The processes for the isolation and purification of antihypercholesterolemic agents disclosed in the earlier 5 patents include a variety of combinations of extraction, chromatography, lactonisation and crystallisation methods. The purity of the final product obtained by these procedures comply with the USP standards but the yields of the desired product are relatively low. In addition, they require both large amounts of organic solvents and the 10 large equipment suited for these quantities.

1 The isolation process disclosed in WO 92/16276 provides the solution for obtaining HMG-CoA reductase inhibitors of purity greater than 99.5% with the use of industrial HPLC (high performance liquid chromatography) equipment.

15 According to WO 92/16276 the crude HMG-CoA reductase inhibitor, with a purity of $\geq 85\%$, is dissolved in an organic solvent or in a solution of organic solvent and water. The mixture is then buffered to a pH between 2 and 9 and placed on the HPLC column. After the HMG-CoA

20 reductase inhibitor peak of interest is collected, a portion of solvent is removed and the water is added or alternatively two-thirds of the solvent mixture are removed and the HMG-CoA reductase inhibitor is crystallised. At the end, the purity of the product

25 obtained by this process is at least 99.5% with the yield of around 90%.

The method disclosed in WO 92/16276 enables obtaining of HMG-CoA reductase inhibitors of high purity, with relatively high yields, the disadvantage of the method 30 over the conventional chromatography columns are relatively small quantities of the substance loaded per HPLC column. Small samples to be fed into the column are also related with increased number of repetitions of the isolation operations in order to obtain sufficient 35 quantities of the desired substance, and consequently

large amount of the solvents used resulting in higher production costs.

Displacement chromatography method, the basis of the present invention, does not substantially differ from 5 previously used chromatography methods.

Displacement chromatography is based on competition of the components of the sample fed into the column for active sites on the stationary phase. Individual components of the sample displace one another like a train, the 10 displacer, having the very high affinity for the stationary phase and travelling behind the fed sample along the column, drives the separation of the sample components into one-compartment zones which move at the same velocity as the displacer. Concentrating of 15 individual components is carried out simultaneously with the purification.

The principle of displacement chromatography method is relatively old as it has been known since 1943 but it was introduced into practice as late as 1981 because of the 20 lack of efficient columns (Cs. Horvath et al., J. Chromatogr., 215 (1981) 295; J. Chromatogr., 330 (1985) 1; J. Chromatogr., 440 (1988) 157). These papers, introduced herein by way of reference, describe the analytic and preparative separation and purification of biologically 25 active peptides and polymyxin antibiotics (polypeptides) using reversed-phase high performance liquid chromatography columns in the displacement mode. For polymyxins octadecyl silica gel columns 250 x 4.6 mm, particle size 5 μ m, 10% acetonitrile in water as the mobile 30 phase and different tetraalkylammonium halogenides as the displacer were used.

In recent investigations in the field of displacement chromatography (S.M. Cramer et al., Enzyme Microb. Technol., 11 (1989) 74; Prep. Chromatogr., 1 (1988) 29; J. Chromatogr., 394 (1987) 305; J. Chromatogr., 439 (1988) 35

341; J. Chromatogr., 454 (1988) 1 (theoretic optimisation)); A. Felinger et al., J. Chromatogr., 609 (1992) 35 (theoretic optimisation), all papers being introduced herein by way of reference) similar columns 5 were used; the mobile phase was methanol in the phosphate buffer, the displacer was 2-(2-t-butoxyethoxy)ethanol (BEE) in acetonitrile and sodium acetate. Different peptides, proteins and cephalosporin C antibiotic were used as the samples.

10 US Pat. No. 5,043,492 (27.08.1991) and EP 416.416, 23 respectively, describe the method for purifying certain low molecular (below 1000 daltons) peptides (in particular, tuftsin and synthetic derivatives thereof) with displacement ion-exchange chromatography where the 15 stationary phase used is cationic-exchange resin, the transporter solvent is water or dilute solutions of a variety of strong acids, and the displacer used is triethylenetetraammonium salt in different concentrations. In US patent application 08/875,422, yet unpublished, the 20 use of displacement chromatography for the isolation and purification of vancomycin is described.

Technical Solution

It is sometimes difficult to obtain the active substance 25 of high purity in a large scale as many technologies applicable to a laboratory scale are not sufficiently economical in large scale production operations to justify use thereof or do not meet the environmental criteria. The above facts compel the industry to search for new technologies 30 that will provide both the high-quality product and the economically and ecologically acceptable production.

The present invention has solved the drawbacks of the processes known from the older patents and other literature as it enables to obtain the pure HMG-CoA

reductase inhibitors and, additionally, the purifying process *per se* is not time-consuming providing high yields, using small amounts of solvents. The process is nature friendly; in addition, it is not demanding in terms 5 of space and energy thus enabling an economical large scale production.

Description of the invention

The present invention provides a process for the 10 purification of HMG-CoA reductase inhibitors employing displacement chromatography. That is, at least one of the steps in the process of the purification of crude HMG-CoA reductase inhibitor includes displacement chromatography.

The HMG-CoA reductase inhibitor to be purified is, for 15 example, selected from the group consisting of mevastatin, pravastatin, lovastatin, simvastatin, fluvastatin and atorvastatin. The selected inhibitor may be in the lactone form or in the form of the acid or the salt thereof for being purified by means of displacement chromatography.

) The displacement chromatography being characteristic for the process of the present invention preferably includes the following steps:

- a) conditioning a chromatography column with an appropriate mobile phase,
- .5 b) feeding the crude HMG-CoA reductase inhibitor dissolved in the mobile phase,
- c) introducing the displacer for displacing the HMG-CoA reductase inhibitor from the column, and
- d) obtaining the purified HMG-CoA reductase inhibitor.

30 The purified HMG-CoA reductase inhibitor is preferably obtained by

- d1) collecting the fractions and

d2) analyzing the fractions with analytical HPLC and pooling the fractions depending on the quality of purity.

After the purified HMG-CoA reductase inhibitor has been obtained, the chromatography column may be regenerated by

5 washing of the column with alcohol/water mixture to elute the displacer.

HMG-CoA reductase inhibitors obtained in the herein-described manner are then isolated from the mobile phase according to the methods already known from the state of

10 prior art, for example by lyophilisation or, preferably, by crystallization to obtain the lactone form, the acid form or the salt form (preferably alkaline or earth alkaline salts) thereof.

15 The fractions containing a considerable percentage of HMG-CoA reductase inhibitors, in addition to impurities, may be re-subjected to the process resulting in the total yield exceeding 95%.

The stationary phase used is a reverse phase where natural (silica gel with alkyl chains of a different length) or

20 synthetic (C-18 or C-8 organic) stationary phases are suitable. Preferably, a synthetic cross-linked polymer matrix of styrene and divinylbenzene is used. The particle size of the stationary phase is suitably from 3 to 20 μm , preferably between 7 and 15 μm .

25 The mobile phase used is preferably selected from water, acetonitrile/water solution and aqueous solutions of lower (preferably C₁-C₄) alcohols, buffered dilute solutions of organic, halogenated organic or inorganic acids, e.g. formic, acetic, propionic, hydrochloric, boric.

30 phosphoric, carbonic or sulphuric acids with cations of alkaline metals, with ammonia or with amines. Water and aqueous solutions with acetonitrile and especially with methanol or ethanol are particularly preferred, and the

35 content of the organic solvent in the aqueous solutions preferably is 80% or below, more preferably 45% or below

and particularly 30% or below. Since toxic methanol in the mobile phase may be replaced by less toxic ethanol, or may be at least partially replaced by water with good results, removal of waste solvents is simpler, therefore, the

5 present invention is a marked improvement compared to the state of prior art judging from the ecological aspect.

The pH of the mobile phase used is preferably between 4.5 and 10.5, more preferably between 6.5 and 8, and particularly around 7. The flow rate of the mobile phase

10 through the column is suitably adjusted to lie between 1.5 and 30 ml/(min cm²), preferably between 3 and 15 ml/(min cm²). At the time when the displacer is introduced into the chromatography column by being mixed with the mobile phase, the flow rate is preferably adjusted to lie between 1.5 and 15 ml/(min cm²) and particularly between 3 and 10 ml/(min cm²), because higher flow rates cause the dilution of the samples to be collected, and also the separation becomes worse.

15 The displacer suitably is a compound having an amphiphilic structure, such as surfactants, detergents and the like.

20 Examples of the displacer are long chain alcohols, long chain carboxylic acids, long chain alkyl ammonium salts, aromatic dicarboxylic acid esters, oxo- and dioxo-alcohols, polyalkylene polyglycol ethers such as

25 diethylene glycol mono- (or di-) alkylethers, polyaryl or polyalkylene polyaryl ethers such as Triton® X-100, etc.

The aforementioned "long chain" means an alkyl chain having at least a C₄-chain, preferably at least a C₁₀-chain and more preferably at least a C₁₄-chain or longer.

30 The concentration of the displacer in the mobile phase is suitably adjusted to be from 1 to 35%, preferably from 2 to 20% and particularly from 7 to 14%.

In the preferred embodiment of controlling the quality of purity in the individual fractions eluted from the

35 chromatography column, an analytical HPLC method directed

to the HMG-CoA reductase inhibitors to be analyzed may be carried out as described in the following.

The sample to be analysed is diluted 100 times with the mobile phase containing 20 mM aqueous NH_4HCO_3 solution with 5 acetonitrile (the proportion of acetonitrile is adjusted such that the retention factor of the analyte is between 5 and 10). 10 μl of this sample is placed on Hypersil ODS column (Hypersil, the United Kingdom, particle size 3 μm , column size 50 \times 4.6 mm) for high performance liquid 10 chromatography. The column is washed with the mobile phase at the flow rate of 2 ml/min. Absorbance is measured at 235 nm. HPLC purity of the sample is calculated from the ratio between the areas of individual peaks in the chromatogram.

15 After completed chromatography the stationary phase is preferably regenerated, for example using the mobile phase with 20 to 100% aqueous solution of lower alcohol.

The invention is illustrated but in no way limited by the following examples.

20

EXAMPLES

Example 1

Crude sodium salt of pravastatin (1.0 g, HPLC purity 88%, assay 85%) was dissolved in 10 ml of the mobile phase A 25 (distilled water), pH was adjusted to 7 with 0.2M aqueous NaOH solution and filtered. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 30 11 μm , column size 250 \times 10 mm. The column was washed with the mobile phase B containing 7% of diethyleneglycol monobutylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 0.5 ml fractions were collected with an initial increase in the

absorbance. When the signal decreased the column was washed with 25 ml of 70% methanol. The obtained fractions were analyzed by the herein above-described HPLC analytical method. The fractions with a purity \geq 99.5% 5 were pooled. In the pooled fractions (7 ml) the HPLC purity was 99.8%.

Example 2

Crude sodium salt of pravastatin (0.4 g, HPLC purity 88%, assay 85%) was dissolved in 5 ml of the mobile phase A 10 (distilled water), pH was adjusted to 7 with 0.2M aqueous NaOH solution and filtered. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Kromasil 100 C-18 column (EKA Chemicals AB, Sweden), particle size 10 μm , column size 200 \times 10 mm. The column was washed with the mobile 15 phase B containing 7% of Triton X-100 in mobile phase A at the flow rate of 1 ml/min. Absorbance was measured at 260 nm, and the 0.5 ml fractions were collected with an initial increase in the absorbance. The obtained fractions 20 were analysed by the above described HPLC analytical method. The fractions with a purity \geq 99.5% were pooled. In the pooled fractions (3 ml) the HPLC purity was 99.7%.

Example 3

25 0.6 g of the crude sodium salt of pravastatin was dissolved in 5 ml of distilled water. The protocol described in Example 1 was used with the exception of the mobile phase used (30% aqueous methanol solution) and the pooled fractions with a HPLC purity of 99.8% were 30 obtained.

Example 4

The method described in Example 3 was repeated wherein the concentration of the displacer in the mobile phase was 14%. In the fractions pooled, according to the criterion 5 described in Example 1, HPLC purity was 99.8%.

Example 5

Pravastatin lacton (0.4g, HPLC purity 85%) was dissolved in 33 ml of the mobil phase A containing 45% methanol. The 10 column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 μm , column size 250 \times 10 mm. The column was washed with the mobile phase B containing 15 2% of diethyleneglycoldibutylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of 70% 20 methanol.

The fractions with a purity \geq 99.5% were pooled. In the pooled fractions the HPLC purity was 99.7%.

25 Example 6

Pravastatin lacton (0.3g, HPLC purity 85%) was dissolved in 80 ml of the mobil phase A containing 30% methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto the 30 Licrosphere RP 18 column, particle size 12 μm , column size 200 \times 10 mm. The column was washed with the mobile phase B containing 5% of diethyleneglycolmono-n-hexylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance

was measured at 235 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of 90% methanol. The obtained fractions were analysed by the 5 above described HPLC analytical method.

The fractions with a purity \geq 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

10 Example 7

Pravastatin lacton (0.3g, HPLC purity 85%) was dissolved in 25 ml of the mobil phase A containing 35% acetonitrile. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto 15 the Licrosphere RP 18 column, particle size 12 μ m, column size 200 \times 10 mm. The column was washed with the mobile phase B containing 1% of diethyleneglycoldibutylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 235 nm, and the 1ml fractions were 20 collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of 90% methanol. The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity \geq 99.5% were pooled. In the 25 pooled fractions the HPLC purity was 99.8%.

Example 8

The method described in Example 7 was repeated wherein the mobile phase B was 0.85% diethylphthalat in the mobile 30 phase A.

The fractions with a purity \geq 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 9

Simvastatin lacton (0.42g, HPLC purity 87%) was dissolved in 6 ml of the 66% acetonitrile and hydrolysed with 1.2mmol of sodium hydroxide. Acetonitrile was removed and 5 pH was adjusted to 7 with diluted H₃PO₄. The column was equilibrated with mobile phase A containing 14% of methanol. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, 10 column size 250 x 10 mm. The column was washed with the mobile phase B containing 6.7% of diethyleneglycolmono-n-hexylether in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 0.5ml fractions were collected with an initial increase in the 15 absorbance. When the signal decreased the column was washed with 25 ml of methanol.

The fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 10

Simvastatin lacton (0.5g, HPLC purity 87 %) was dissolved in 20 ml of the mobile phase containing 70% of methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto 25 the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 µm, column size 250 x 10 mm. The column was washed with the mobile phase B containing 3% of decanoic acid in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 30 0.75 ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol. The obtained fractions were analyzed by the herein above described method. The 35 fractions with a purity ≥ 99.5% were pooled. In the pooled fractions the HPLC purity was 99.7%.

Example 11

Simvastatin lacton (0.5 g, HPLC purity 87%) was dissolved in 20 ml of the mobile phase containing of 60% acetonitrile. The column was equilibrated with mobile 5 phase A. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 μm , column size 250 \times 10 mm. The column was washed with the mobile phase B containing 2% of tetrakis(decyl)amonium 10 bromide in mobile phase A at the flow rate of 4.5 ml/min. Absorbance was measured at 260 nm, and the 1ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

15 The fractions with a purity \geq 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 12

20 Lovastatin lacton (0.5g, HPLC purity 87%) was dissolved in 60 ml of the 75% methanol. The column was equilibrated with mobile phase A containing 70% of methanol. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, 25 Germany), particle size 11 μm , column size 250 \times 10 mm. The column was washed with the mobile phase B containing 70% of methanol and 4.5% of decanoic acid in mobile phase A at the flow rate of 6 ml/min. Absorbance was measured at 260 nm, and the 1ml fractions were collected with an 30 initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol. The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity \geq 99.5% were pooled. In the pooled fractions the HPLC purity was 99.9%.

Example 13

- 5 Lovastatin lacton (0.42g, HPLC purity 87 %) was dissolved in 8 ml of the 50% acetonitrile and hydrolysed with 1.5 mmol of sodium hydroxide. Acetonitrile was removed and pH was adjusted to 7 with diluted H_3PO_4 . The column was equilibrated with mobile phase A containing 14% of
- 10 methanol. The sample obtained in the above-described manner was fed onto the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 μm , column size 250 \times 10 mm. The column was washed with the mobile phase B containing 6.7% of diethyleneglycolmono-n-
- 15 hexylether in mobile phase A at the flow rate of 1 ml/min. Absorbance was measured at 260 nm, and the 0.25 ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.
- 20 The obtained fractions were analysed by the method described in example 9. The fractions with a purity \geq 99.5% were pooled. In the pooled fractions the HPLC purity was 99.8%.

Example 14

- 25 Mevastatin lacton (0.5g, HPLC purity 85%) was dissolved in 150 ml of the mobile phase A containing 70% of methanol. The column was equilibrated with mobile phase A. The sample obtained in the above-described manner was fed onto
- 30 the Grom-Sil 120-ODS HE column (Grom Analytic + HPLC GmbH, Germany), particle size 11 μm , column size 250 \times 10 mm. The column was washed with the mobile phase B containing 4.5% of decanoic acid in mobile phase A at the flow rate of 6 ml/min. Absorbance was measured at 260 nm, and the

1 ml fractions were collected with an initial increase in the absorbance. When the signal decreased the column was washed with 25 ml of methanol.

5 The obtained fractions were analysed by the above described HPLC analytical method.

The fractions with a purity $\geq 99.5\%$ were pooled. In the pooled fractions the HPLC purity was 99.8%.

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